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Clonality of Acquired Primary Pure Red Cell Aplasia

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Acquired primary pure red cell aplasia (PRCA) has frequently been shown to be associated with T cells that inhibit marrow erythropoiesis. A 41-year-old female presented with anemia in December 1985. Bone marrow examination revealed 1.8% erythroid cells. A diagnosis of PRCA was made. She received prednisolone, and her hemoglobin level recovered to 12 g/dl. In February 1995, her hemoglobin level decreased to 5.8 g/dl, and acquired primary PRCA recurred. Surface markers of peripheral blood mononuclear cells demonstrated CD4/8 ratio inversion. The T-cell receptor (TCR)-β chain gene showed germ line configuration by Southern blot analysis of the mononuclear cells in peripheral blood. However, stepdown polymerase chain reaction analysis revealed that the TCR-β gene of peripheral blood mononuclear cells was rearranged. With highly sensitive polymerase chain reaction analysis, clonality of T cells was confirmed. We propose that some acquired primary PRCA patients have a T-cell clonal disorder, similar to some PRCA patients with large granular lymphocytes leukemia or thymoma. Am. J. Hematol. 62:193–195, 1999. © 1999 Wiley-Liss, Inc.

Key words: pure red cell aplasia; T-cell receptor-β; cyclosporin-A

INTRODUCTION

Pure red cell aplasia (PRCA) is characterized by anemia, reticulocytopenia, and severe erythroid hypoplasia of bone marrow. Acquired secondary PRCA has been described in association with numerous conditions such as thymoma, hematological malignancies, non-hematological solid tumors, infections, drugs, exposure to chemicals, hemolytic anemias, collagen disease, pregnancy, severe renal failure, and severe nutritional deficiencies [1]. Acquired primary PRCA has frequently been shown to be associated with immunoglobulins [2] or T cells [3] that inhibit marrow erythropoiesis. We found that a case of acquired primary PRCA was a clonal disease of T cells by the polymerase chain reaction (PCR) method.

CASE REPORT

A 41-year-old female presented with anemia in December 1985. Laboratory findings were as follows: red blood cell count 2.75×10^{12}/l, hemoglobin 9.3 g/dl, hematocrit 27.8%, leucocytes 6×10^{9}/l, platelets 20×10^{10}/l and 0.3% reticulocytes. In May 1986, her hemoglobin level decreased to 6.2 g/dl. Subsequent bone marrow examination revealed 1.8% erythroid cells. A diagnosis of PRCA was made. She received prednisolone (1 mg/kg/day). Four weeks after the initiation of prednisolone, her hemoglobin level recovered to 12 g/dl. With maintenance doses of prednisolone (2.5–5 mg/day), her hemoglobin levels were maintained at 11–12 g/dl for 10 years. In February 1995, her hemoglobin level decreased to 5.8 g/dl, and acquired primary PRCA recurred. She visited our department with anemia in March 1995. She received a red blood cell transfusion (6 units, 1 unit derived from 200 ml of whole blood). Laboratory findings were as follows: red blood cell count 1.24×10^{12}/l, hemoglobin 5.0 g/dl, hematocrit 14.2%, platelets 24.8×10^{10}/l, leucocytes 4.2×10^{9}/l with 83.5% neutrophils, 12.5% lymphocytes, 4.0% monocytes, and 0.2% reticulocytes. Bone marrow aspirate smears and biopsy sections revealed normal myeloid and megakaryocyte differentiation with few erythroid precursors. Chromosomal analysis showed no abnormalities. The granular lymphocytes (GL) were not increased. Surface markers of pe-

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ripheral blood mononuclear cells demonstrated the following: CD1 0.1%; CD2 85.3%; CD3 89.8%; CD4 22.2%; CD5 49.8%; CD7 73.4%; CD8 53.6%; CD10 0.1%; CD13 1.4%; CD14 0.5%; CD16 6.1%; CD19 1.5%; CD20 1.2%; CD25 1.6%; CD33 3.3%; CD57 14.0%; CD118 24.3%; HLA-DR 32.5%. The CD4/8 ratio was inverted. The T-cell receptor (TCR)-β chain gene showed germ line configuration by Southern blot analysis of mononuclear cells in the peripheral blood. A PCR test for Parvovirus B19 was negative. The patient's serum did not show circulating auto-antibodies, antibodies to human T-cell leukemia virus-1, or human immunodeficiency virus. Chest computed tomography did not reveal thymoma. A diagnosis of acquired primary PRCA was made. Doses of prednisolone were increased to 1 mg/kg/day. After 2 months of prednisolone, her hemoglobin level recovered to 11 g/dl. When prednisolone was reduced to 0.12 mg/k day, anemia recurred. Therapy with cyclosporin-A at 6 mg/kg/day was started in January 1997. Four weeks after initiation of cyclosporin-A therapy, the patient developed reticulocytosis.

In July 1998, we performed TCR-β gene analysis of peripheral blood mononuclear cells by Southern blot and PCR assays. The TCR-β chain gene showed germ line configuration by Southern blot analysis. However, stepdown PCR analysis [4] revealed that the TCR-β gene of peripheral blood mononuclear cells was rearranged (Fig. 1) [5]. Thus, with highly sensitive analysis of PCR, acquired primary PRCA revealed T-cell clonal disease. All treatments were approved by the Investigational Review Board of Tokyo Women's Medical University, and written informed consent was obtained. Bone marrow aspirates, and peripheral blood were obtained after informed consent according to the guidelines of the Investigational Review Board of Tokyo Women's Medical University.

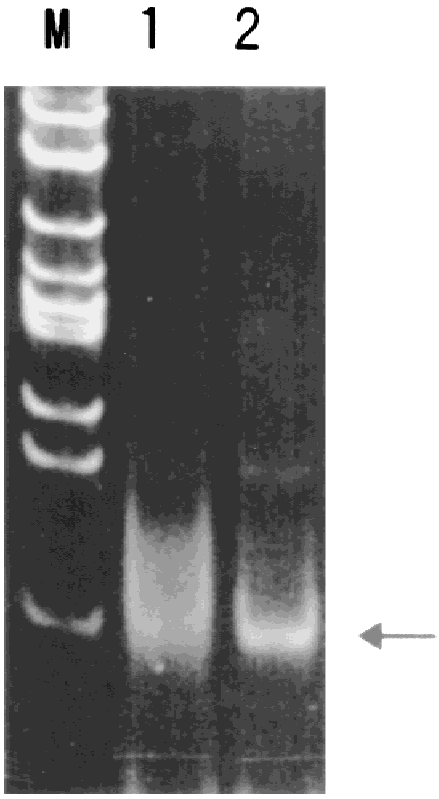

Fig. 1. Detection of clonal TCR-β gene rearrangement of peripheral blood mononuclear cells by stepdown PCR analysis. Sequences of primers were as the follows: V 5′-TGTATCTCTGTGCCAGCAG-3′, D1 5′-CAAAGCTGTAACAT-TGTGGGGAC-3′, D2 5′-TCATGGTGTAACATTGTGGGGAC-3′, J1 5′-ACAGTGAGCCGGGTTCC-3′, J2 5′-AGCACCGTG-AGCCTGGTGCC-3′. M, molecular size marker. Lane 1, negative control (healthy donor's DNA 0.1 μg). Lane 2, a patient with acquired primary PRCA. The position of the rearranged fragment is marked by an arrow.

DISCUSSION

Mamiya et al. reviewed 115 patients with acquired PRCA in Japan and found that 51 patients (44%) with no identifiable underlying disease were classified as having the primary form [6]. They noted that some patients with acquired PRCA showed a reversed CD4/8 ratio in peripheral blood lymphocytes. In our patient, PRCA was caused by a clonal disorder of T cells.

We could not determine the clonality of T cells by Southern blot analysis in this case. However, with highly sensitive PCR analysis, we confirmed the clonality of T cells. This analysis detected 5–10 clonal cells per 10^4 nucleated cells [7]. Underlying diseases of acquired secondary PRCA with clonal T cells include large granular lymphocyte (LGL) leukemia and thymoma. LGL leukemia is a clonal disorder with TCR rearrangement, and is often associated with PRCA [8]. We previously reported a patient with PRCA accompanied by thymoma who presented neoplastic proliferation of $CD8^+$ T cells in the peripheral blood and thymus with monoclonal rearrangement of the TCR-β chain gene [9]. In some patients with acquired primary PRCA, PRCA with LGL leukemia, and PRCA with thymoma, the clonal T lymphocytes inhibited erythroid precursor cells.

Handgretinger et al. reported a patient with PRCA and a clonal population of LGLs of the γ/δ T-cell type [10]. They speculated that the cytotoxic γ/δ T cells inhibited erythroid precursor cells by signaling through killer-cell inhibitory receptors. Although most LGL cells were the TCRα/β-type, some α/β T cells express the killer-cell inhibitory receptor. Because the present case showed monoclonal rearrangement of the TCR-β chain gene, the mechanism of erythroid cell inhibition might be different from γ/δ T cell as PRCA with LGL leukemia. We propose that some acquired primary PRCA patients have a T-cell clonal disorder, similar to some PRCA patients with LGL leukemia and thymoma.

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